

tetrapods, wherein only *Hoxa2* acts as a selector gene, and in zebrafish, in which both *hoxa2b* and *hoxb2a* function redundantly. To understand whether the functional redundancy of zebrafish *hoxa2b* and *hoxb2a* is a common characteristic of teleosts, our laboratory has been conducting experiments to examine the expression patterns and genetic function of *Hox* PG2 genes among evolutionarily divergent teleosts, specifically those with three genes (*hoxa2a*, *hoxa2b*, and *hoxb2a*). Whole mount *in situ* hybridization data for striped bass and Nile tilapia have shown that, while all three *Hox* PG2 genes are expressed in PA2, only *hoxa2a* expression is maintained in PA2 during arch chondrogenesis. By contrast, in the Japanese medaka both *hoxa2a* and *hoxb2a* are expressed into the chondrogenic phase of PA2 development, while *hoxa2b* is expressed weakly and not at all during the chondrogenic phase. These results suggest that *hoxa2a* alone specifies the identity of PA2 in striped bass and tilapia, while both *hoxa2a* and *hoxb2a* act together or redundantly as selector genes in medaka. While functional studies will be required to determine the precise role of medaka *hoxa2a* and *hoxb2a* in PA2 patterning, preliminary *Hox* PG2 gene knockdown results in tilapia suggest that *hoxa2a* acts alone as a selector gene of PA2 identity.

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Amphioxus *AmphiDelta*: Evolution of delta protein structure, segmentation, and neurogenesis

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The basal chordate amphioxus has a single *Delta* gene, *AmphiDelta* that codes for a single-pass membrane protein with nine EGF repeats in the extracellular domain. Previous phylogenetic analyses of Delta proteins have been inconclusive because of the poor conservation of many non-cysteine amino acids in the EGF repeats and because of independent deletions of major regions. To elucidate the evolution of *Delta* genes we used these deletions plus the numbers of amino acids separating successive cysteines. Comparisons between amphioxus and other animals indicate that *AmphiDelta* retains

features of a basal bilaterian Delta protein-nine EGF repeats and characteristic numbers of amino acids separating successive cysteines. During development, *AmphiDelta* is expressed in the forming somites, pharyngeal endoderm and scattered cells (presumably differentiating neurons) in the neural plate and ectoderm. Expression is strongly associated with cells initiating movements to separate themselves from parent epithelia, either en masse by evagination or by delamination as isolated cells. The *AmphiDelta*-expressing cells delaminating from the ectoderm apparently migrate beneath it as they begin differentiating into probable sensory neurons, suggesting a scenario for the evolutionary origin of vertebrate neurogenic placodes and cranial ganglia.

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Opposite roles of Puf RNA-binding proteins in convergently evolved hermaphrodites

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Caenorhabditis elegans and *C. briggsae* evolved self-fertile hermaphrodites from XX female ancestors (Haag and Doty 2005, PLoS Biology 3: e21). However, they regulate XX spermatogenesis via distinct germline modifications of the core sex determination pathway (Hill et al., 2006, Dev Cell 10:531). Most germline-specific sex determination genes in *C. elegans* are RNA-binding proteins, so we used reverse genetics to study the Puf family of translation repressors in *C. briggsae*. In *C. elegans*, the recently duplicated Puf genes *fbf-1* and *fbf-2* are required for the sperm-oocyte switch in hermaphrodites and act by repressing *fem-3* (Zhang et al., 1997, Nature 390: 477). They are also redundantly required for robust germline proliferation (Crittenden et al., 2002, Nature 417: 660). The closest *C. briggsae* relatives of *fbf-1/2* are three species-specific paralogs, *Cb-puf-1*, *Cb-puf-2* and *Cb-puf-12* (Lamont et al., 2004, Dev Cell 7: 697). *Cb-puf-2*(RNAi) causes hermaphrodite-specific germline feminization, a phenotype opposite that of *fbf-1/2* (RNAi). Triple knockdown of *Cb-puf-1/2/12* produces a germline proliferation defect reminiscent of the *fbf-1/2* double mutant. We propose that the *fbf*-related Puf subfamily has a conserved role in germline proliferation, but acquired distinct roles in sex determination during the independent evolution of hermaphroditism. The latter may be mediated by different target mRNAs, but the conservation of the FBF binding site in the *C. briggsae* *fem-3* 3' UTR (Haag et al., 2002, Curr Biol 12: 2035) suggests that cofactors, such as *nos-3*, may also play key roles in the evolution of translational controls.

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